

received and placed with the Application file. Further, Applicants wish to note that under U.S. practice, the present application is entitled to have the International Filing date of March 13, 1997 as the U.S. filing date for the determination of prior art.

In this regard, Applicants have submitted along with the present response, a verified English translation of the priority document upon which the claim of priority is based. Applicants respectfully request acknowledgment of receipt of said certified copy at the Examiner's convenience.

Claims 2-5 have been cancelled without prejudice, claims 1, 6 and 7 have been amended, and new claims 8-17 have been added. The claim amendments have been effected to put the claims in better form under U.S. practice and to overcome the rejections under 35 USC § 112, 102 and 103. Applicants wish to note that unless otherwise specifically recited below, the claim amendment are merely editorial in nature and should not be construed to limit the scope of the claims. Support for the claim amendments and new claims is readily apparent from the teachings of the specification and the original claims. For specific support, Applicants would like to direct the Examiner's attention to pages 5-11 of the specification.

With regard to the objection of claims 5-7 under 37 CFR 1.75(c) as set forth in item 4 of the Official Action, this objection has been overcome by the cancellation of claim 5 and the amendments to claims 6 and 7. Specifically, Applicants have amended claims 6 and 7 to independent form. Thus, since claims 6 and 7 no longer depend on any claim, this rejection should be withdrawn.

With regard to the objection of claim 3 under 37 CFR 1.75(c) as set forth in item 5 of the Official Action, this objection has been overcome by the cancellation of claim 3. However, Applicants wish to note that claim 3 depended on original claim 1 and not claim 2 as noted by the Examiner. Thus, Applicants believe that this objection of claim 3 was improper and that the cancellation of claim 3 should not prejudice the scope of the claims in any way.

With regard to the rejections of claims 1 and 4 under 35 USC § 112, first paragraph, as set forth in items 7 and 8 of the Official Action, these rejections have been overcome by the cancellation of claim 4, and the wording of the amended claim 1. Specifically, claim 1 has been amended to direct to partial peptides of SEQ ID NO. 1 which the Examiner has indicated to satisfy the requirements of 35 USC § 112, first paragraph. Thus, in light of Applicants' amendment to claim 1, these rejections can no longer be sustained and should be withdrawn.

With regard to the rejection of claims 1-4 under 35 USC § 102(b) as being anticipated by Vandermeeren et al. (J. of Neurochemistry 61:1828-34, 1993), this rejection is deemed to be untenable in view of the wording of the amended claims and is thus respectfully traversed.

To constitute anticipation of the claimed invention, a single prior art reference must disclose each and every material element of the claim. Here, in this case, Vandermeeren et al. fail to teach or suggest the antibody specificity and the partial peptides of the present claims.

The antibody of the present invention, as described in the amended claim 1, is obtained by using, as an immunogen, a partial peptide comprising two amino acid residues at the phosphorylation sites of phosphorylated tau protein in a paired helical filament and plural amino acid residues before and/or after the phosphorylation sites of amino acid sequence of SEQ ID

NO: 1, wherein the two amino acid residues are threonine at position 231 and serine at position 235, or serine at position 412 and serine at position 413 of amino acid sequence of SEQ ID NO: 1.

On the other hand, the antibody AT8 of Vandermeeren et al. is obtained by using, as an immunogen, phosphorylated tau protein as a whole. Furthermore, the obtained antibody AT8 recognizes serine at position 202 and threonine at position 205 based on the teachings of M. Goedert et al. Applicants wish to note that although Vandermeeren et al. disclose that the antibody AT8 recognizes the serines at positions 199-202, the antibodies actually recognizes serine at 202 and threonine at position 205.

Thus, the antibody of the present invention differs from the antibody AT8 of Vandermeeren et al. with respect to the way the antibody is produced and the antibody's specificity to the various phosphorylated sites of the phosphorylated tau protein.

The Examiner states that Vandermeeren et al. teach that the sandwich assay utilizes monoclonal antibody AT8 which recognizes abnormally phosphorylated serines at positions 199-202 in the tau protein.

However, the detection limit for  $\tau$  was less than 5 pg/ml of CSF using AT8, and Vandermeeren et al. disclose that "when a pool of AD CSF samples was concentrated 12 times, resulting in a hypothetical sensitivity of less than 3 pg/ml PHF- $\tau$ , no signal was found. Thus, if PHF- $\tau$ , as detected by the AT8 immunoassay, is present in CSF, its concentration must probably be below 3pg/ml." (see page 1831, right column, lines 11-16, of the reference).

Although Vandermeeren et al. disclose a sensitive sandwich ELISA using AT120, AT120 reacts with both phosphorylated and dephosphorylated PHF  $\tau$ , indicating that its recognizing site is not the phosphorylated site, as described in Fig. 2. The reactivity of the AT120 antibody with PHF- $\tau$  was not sensitive to phosphatase treatment either in ELISA (Fig. 2) or on western blots (see page 1829, right column, lines 4-7 from the bottom, of the reference). Furthermore, according to Fig. 4, AT120 reacts with CFS samples from not only the patients of Alzheimer's disease but also the patients suffering from other neurological diseases (OND). Thus, the detection method using AT120 of Vandermeeren et al. is not specific to Alzheimer's disease.

Conversely, the presently claimed antibodies are specific for Alzheimer's disease as set forth in the Examples of the specification. The claimed antibodies can be used to detect Alzheimer's disease by examining the reactivity of these antibodies with a body fluid sample from an individual suspected of having Alzheimer's disease. The antibodies of Vandermeeren et al., on the other hand, cannot be used in such a method.

Thus, in light of the above, Applicants believe that this rejection can not be sustained and should be withdrawn.

With regard to the rejections of claims 1-4 under 35 USC § 102(b) as being anticipated by Kimura et al. (Dementia, 7:177-81, 1996) or Yamaguchi et al. (Acta Neuropathol., 92:232-241, 1996), these rejections are deemed to be untenable and are thus respectfully traversed.

As noted earlier, the certified copy of the priority document is present in the file and thus, the U.S. filing for the present application is March 13, 1997. Further, with the filing of the

verified English translation of the certified priority document, Applicants' claim of priority has been perfected and the present application is entitled to a priority date of March 13, 1996.

Applicants note that the cited references, Kimura et al. and Yamaguchi et al., were published after the priority date (March 13, 1996) of the present application. Applicants have submitted a catalog showing the month of publication for these references. For Kimura et al., the publication date appears to be around August, 1996 and for Yamaguchi et al., the publication date was in July-August, 1996. Since both of these references have a publication date after March 13, 1996, Applicants submit that Kimura et al. and Yamaguchi et al. are not valid prior art references under 35 USC § 102 and thus, these rejections in view of these references cannot be sustained and should be withdrawn.

With regard to the rejection of claims 1-4 under 35 USC § 102(b) as being anticipated by Biernat et al. (EMBO J., 11(4):1593-97, 1992), this rejection is deemed to be untenable and is thus, respectfully traversed.

Like Vandermeeren et al., the antibody AT8 of Biernat et al. is obtained by using, as an immunogen peptide, the whole phosphorylated tau protein, and the obtained antibody AT8 recognizes serine at position 202 and threonine at position 205.

Thus, the antibody of the present invention differs from the antibody AT8 of Biernat et al. in both the way the antibody is produced and the antibody's specificity to the phosphorylated sites of phosphorylated tau protein.

As the Examiner mentioned, Biernat et al. teach that the switch of tau protein to an Alzheimer-like state includes the phosphorylation of two serine-proline motifs upstream of the

microtubule binding region. However, it does not describe or suggest the method for detecting Alzheimer's disease by examining reactivity of the antibodies by using, as an immunogen, a partial peptide containing a phosphorylated site of phosphorylated tau protein in the PHF, with a body fluid sample from an individual suspected of Alzheimer's disease.

Thus, in view of the above, Applicants respectfully request that this rejection be withdrawn.

With regard to the rejection of claims 1-4 under 35 USC § 102(b) as being anticipated by Takahashi et al. (J of Neurochemistry, 64:1759-68), this rejection is deemed to be untenable and is thus, respectfully traversed.

The antibody of Takahashi et al. is directed against phosphorylated serine at position 199 or phosphorylated serine at position 396. Thus, the antibody of the present invention differs from that of Takahashi et al. with respect to its specificity to the phosphorylation sites of phosphorylated tau protein.

Further, although the Examiner states that Takahashi et al. teach antiserum PS199 antibodies directed to phosphorylated Serine 199 and analysis of immunoreactivity in rat brain, it does not teach or suggest the method for detecting Alzheimer's disease by using an antibody that specifically recognizes phosphorylated tau protein of a body fluid sample from an individual suspected of Alzheimer's disease.

Thus, for the same reasons as Vandermeeren et al. and Biernat et al., this rejection should be withdrawn.

Applicants wish to note that although Vandermeeren et al. (1993) and Biernat et al. (1992) indicate that AT8 recognizes abnormally phosphorylated serines at positions 199-202, Goedert et al. (Neuroscience Letters 189 (1995) 167-170) teach that AT8 recognizes the tau protein phosphorylated at both serine 202 and threonine 205. This teaching is significant since the reference, Goedert et al., was published (1995) after Vandermeeren et al. (1993) and Biernat et al. (1992).

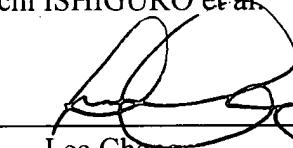
Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made."

In view of the foregoing amendments and remarks, it is respectfully submitted that the Application is now in condition for allowance. Such action is thus respectfully solicited.

If, however, the Examiner has any suggestions for expediting allowance of the application or believes that direct communication with Applicants' attorney will advance the prosecution of this case, the Examiner is invited to contact the undersigned at the telephone number below.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Page 1, line 1, the heading has been deleted in its entirety as follows: [DESCRIPTION];

line 5, the heading "Technical Field" has been replaced with the following:

--Background of the Invention

1. Field of the Invention--;

line 14, the heading "Background Art" has been replaced with --2. Description of the Related Art--.

Page 2, the paragraph beginning at line 19 has been amended as follows:

Tau protein is composed of a group of protein isoforms that usually produce several bands at the molecular weight of 48 to 65 kD as a result of SDS-polyacrylamide gel electrophoresis and it is known to promote formation of microtubule. Tau protein incorporated in the PHF of the Alzheimer diseased brain was proved to be abnormally phosphorylated as compared with that in the normal brain using polyclonal antibody to PHF (anti-ptau; J. Biochem., 99, 1807-1810 (1986)) and monoclonal antibody to tau protein (tau-1 antibody; Proc. Natl. Acad. Sci. USA, 83, 4913-4917 (1986)). The phosphorylation sites of phosphorylated tau protein incorporated in the PHF were also indentified (JP 6-239893 A). Thus, functions of tau protein involved in Alzheimer's disease [has] are being clarified.

Page 3, line 13, the heading "Disclosure of the Invention" has been replaced with --

Summary of the Invention--

Page 5, the paragraph beginning at line 6 has been amended as follows:

Yet another embodiment of this invention provides methods for detecting Alzheimer's disease [comprises] comprising examining reactive reactivity between any one of the above-described antibodies and a sample from an individual suspected of Alzheimer's disease.

above line 11, insert the following new heading --Description of the Preferred

Embodiments--

Paragraphs from page 11, line 20 to page 12, line 1

Brief Description of the Drawings

Figure 1 is the dot blot showing specificity of the antibodies obtained by immunization with a partial peptide containing a phosphorylation site of phosphorylated tau protein.

Figure 2 is photographs of electrophoresis (immunoblotting) showing reactivity of the TS fraction (the fraction obtained by removing IgG from the supernatant of human cerebral cortex suspension) obtained in Example with the antibodies used in the present invention.

Figure 3 is photographs of electrophoresis (immunoblotting) showing reactivity of the SDS precipitation fraction obtained in Example with the antibodies used in the present invention.

Figure 4 is photographs of electrophoresis (immunoblotting) showing reactivity of the SDS precipitation fraction obtained in Example with the antibodies used in the present invention.

Figure 5 shows a calibration curve in the competitive RIA obtained in Example.

Figure 6 shows the results of measuring the concentrations of phosphorylated tau protein in the cerebrospinal fluid from patients with Alzheimer's disease and patients with no dementia obtained in Example.



Page 12, line 21, delete the heading “Best Mode for Carrying out the Invention”;

the paragraph beginning at line 22 has been amended as follows:

The present invention will now be described below in more detail with reference to Examples, but is not construed to be limited thereto.

Page 40, line 15, delete the heading “Industrial Applicability”.



**In the Claims:**

1. (Amended) [Antibodies] An antibody obtained by using, as an immunogen, a partial peptide comprising two amino acid residues at the phosphorylation sites of phosphorylated tau protein in a paired helical filament and plural amino acid residues before and/or after the phosphorylation sites of amino acid sequence of SEQ ID NO: 1, wherein the two amino acid residues are threonine at position 231 and serine at position 235, or serine at position 412 and serine at position 413 of amino acid sequence of SEQ ID NO: 1.

6. (Amended) A reagent kit [used] for detecting Alzheimer's disease, comprising [at least] one or more [the] antibodies [as defined in any one of claims 1 to 5] obtained by using, as an immunogen, a partial peptide comprising one or more amino acid residue(s) at the phosphorylation sites of phosphorylated tau protein in a paired helical filament and plural amino acid residues before and/or after the phosphorylation site(s) of amino acid sequence of SEQ ID NO: 1, wherein the phosphorylation site(s) are one or more amino acid residue(s) selected from the group consisting of serine at position 198, serine at position 199, threonine at position 231, serine at position 235, serine at position 262, serine at position 396, threonine at position 403, serine at position 404, serine at position 409, serine at position 412, serine at position 413, and

serine at position 422 of amino acid sequence of SEQ ID NO: 1.



7. (Amended) [Methods] A method for detecting Alzheimer's disease, comprising [which are characterized by] examining reactivity of one or more [the] antibodies[, as defined in any one of claims 1 to 5,] with a body fluid sample from an individual suspected of Alzheimer's disease, wherein said antibodies are obtained by using, as an immunogen, a partial peptide comprising one or more amino acid residue(s) at the phosphorylation sites of phosphorylated tau protein in a paired helical filament and plural amino acid residues before and/or after the phosphorylation site(s) of amino acid sequence of SEQ ID NO:1, wherein the phosphorylation site(s) are one or more amino acid residue(s) selected from the group consisting of serine at position 198, serine at position 199, threonine at position 231, serine at position 235, serine at position 262, serine at position 396, threonine at position 403, serine at position 404, serine at position 409, serine at position 412, serine at position 413, and serine at position 422 of amino acid sequence of SEQ ID NO: 1.